

### LISTING OF CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (currently amended) An immunogenic conjugate comprising:

a protein and either a an isolated polysaccharide or an isolated oligosaccharide,

wherein the protein is a bacterial or non-naturally occurring synthetic protein that comprises at least one lysine residue or at least one cysteine residue

wherein the polysaccharide or the oligosaccharide has been N-deacetylated and N-acryloylated to comprise at least one N-acryloyl group,

wherein the N-deacetylated and N-acryloylated polysaccharide or the N-deacetylated and N-acryloylated oligosaccharide is covalently attached to the protein via a  $\beta$ -propionamido linkage between the at least one lysine residue or the at least one cysteine residue and the at least one N-acryloyl group of the polysaccharide or the oligosaccharide,

wherein the polysaccharide or the oligosaccharide is isolated ~~derived~~ from bacteria, yeast, or cancer cells, and

wherein the polysaccharide or the oligosaccharide is natural or synthetic.

2. (cancelled)

3. (cancelled)

4. (currently amended) The conjugate according to claim 1 wherein the polysaccharide or the oligosaccharide is isolated ~~derived~~ from *Escherichia coli*, Meningococcus, Pncumococcus, ~~Streptococcus~~, Streptococcus, ~~Neisseria~~, Neisseria, ~~Salmonella~~, Salmonella, ~~Klebsiella~~, Klebsiella, or ~~Pseudomonas~~ Pseudomonas.
5. (currently amended) The conjugate according to claim 1 wherein the polysaccharide or the oligosaccharide is isolated ~~derived~~ from Group B *Streptococcus* selected from the group consisting of type Ia, type Ib, type II, type III, type V, type VIII, and combinations thereof.
6. (currently amended) The conjugate according to claim 4 wherein the polysaccharide or the oligosaccharide is isolated ~~derived~~ from a Meningococcus group selected from the group consisting of group B, group C, group Y, group W135, and combinations thereof.
7. (currently amended) The conjugate according to claim 4 wherein the polysaccharide or the oligosaccharide is isolated ~~derived~~ from *E. coli* K1, *E. coli* K92, Pneumococcus type 4, Pneumococcus type 14, *Streptococcus* group A, *Streptococcus* group C, or combinations thereof.
8. (previously presented) The conjugate according to claim 1 wherein the protein is selected from the group consisting of tetanus toxoid, diphtheria toxoid, a *Neisseria meningitidis* outer membrane protein, pneumolysoid, C- $\beta$  protein from group B *Streptococcus* and non-IgA-binding C- $\beta$  protein from group B *Streptococcus*.

9. (previously presented) The conjugate according to claim 8 wherein the protein is recombinantly produced.
10. (withdrawn) The conjugate according to claim 9 wherein the protein is recombinant *N. meningitidis* outer membrane protein.
11. (previously presented) The conjugate according to claim 1 wherein the polysaccharide or the oligosaccharide comprises a glycosaminoglycan.
12. (previously presented) The conjugate according to claim 1 wherein the polysaccharide or the oligosaccharide comprises glycosyl residues of a structural repeating unit having at least one free amino group or N-acyl group.
13. (previously presented) The conjugate according to claim 12 wherein the glycosyl residue is selected from the group consisting of glucosamine, galactosamine, mannosamine, fucosamine and sialic acid.
14. (cancelled)
15. (currently amended) The conjugate according to claim 1 wherein the polysaccharide or the oligosaccharide is isolated ~~derived~~ from Group B *Streptococcus* type III, and wherein the protein is tetanus toxoid.

16. (currently amended) An immunogenic conjugate produced by a method comprising:

A) removing at least 50% ~~about 50%~~ of N-acetyl groups from either ~~a~~ an isolated polysaccharide or an isolated oligosaccharide to form an N-deacetylated polysaccharide or an N-deacetylated oligosaccharide, wherein the polysaccharide or the oligosaccharide is isolated ~~derived~~ from bacteria, yeast, or cancer cells, and wherein the polysaccharide or the oligosaccharide is natural or synthetic,

B) adding an acryloylating reagent to the N-deacetylated polysaccharide or the N-deacetylated oligosaccharide to replace at least one removed N-acetyl group with at least one N-acryloyl group to form an N-acryloylated polysaccharide or an N-acryloylated oligosaccharide, and

C) coupling the N-acryloylated polysaccharide or the N-acryloylated oligosaccharide with a bacterial or non-naturally occurring synthetic protein comprising at least one lysine residue or at least one cysteine residue to form a  $\beta$ -propiionamido linkage between the at least one lysine residue or the at least one cysteine residue of the protein and the at least one N-acryloyl group of the N-acryloylated polysaccharide or the N-acryloylated oligosaccharide.

17. (cancelled)

18. (previously presented) The conjugate according to claim 16 wherein the N-acryloylated polysaccharide or the N-acryloylated oligosaccharide is coupled with the protein at a pH of about 7.0.

19. (previously presented) The conjugate according to claim 16 wherein the N-acryloylated polysaccharide or the N-acryloylated oligosaccharide is coupled with the protein at a pH above 9.
20. (previously presented) The conjugate according to claim 16 wherein the N-acryloylated polysaccharide or the N-acryloylated oligosaccharide is coupled with the protein in a reagent selected from the group consisting of phosphate buffer, bicarbonate buffer, and borate buffer.
21. (previously presented) The conjugate according to claim 16 wherein the N-acetyl groups are removed by a base or an enzyme and the acryloylating reagent is selected from the group consisting of N-acryloyl chloride, acryloyl anhydride, acrylic acid and a dehydrating agent.
22. (previously presented) A pharmaceutical composition comprising the conjugate according to any one of claim 1 and claim 16 and a pharmaceutically acceptable carrier.
23. (original) The pharmaceutical composition according to claim 22 further comprising an adjuvant.
24. (original) The pharmaceutical composition according to claim 23 wherein the adjuvant is selected from the group consisting of alum and stearyl tyrosine.

25. (previously presented) The pharmaceutical composition according to claim 22 further comprising a second immunogenic component, said second immunogenic component selected from the group of immunogens consisting of diphtheria-tetanus-pertussis (DTP), diphtheria-tetanus-acellular pertussis (DTaP), tetanus-diphtheria (Td), diphtheria-tetanus-acellular pertussis-*Haemophilus influenzae type b* (DTaP-Hib), diphtheria-tetanus-acellular pertussis-inactivated poliovirus-*Haemophilus influenzae type b* (DTaP-IPV-Hib), and combinations thereof.
26. (previously presented) The immunogenic conjugate according to any one of claim 1 and claim 16, wherein the conjugate elicits an immune response specific to the polysaccharide or the oligosaccharide.
27. (previously presented) The immunogenic conjugate according to claim 26, wherein the immune response is generation of an immunoglobulin specific to the polysaccharide or the oligosaccharide.
28. (previously presented) The immunogenic conjugate according to claim 27 wherein the immunoglobulin is IgG, IgM, IgA or combinations thereof.
29. (withdrawn) A method of making a  $\beta$ -propionamido-linked polysaccharide-protein conjugate or a  $\beta$ -propionamido-linked oligosaccharide-protein conjugate comprising:

- A) de-N-acetylating a polysaccharide or an oligosaccharide using a de-N-acetylating reagent to form a de-N-acetylated polysaccharide or de-N-acetylated oligosaccharide,
- B) N-acryloylating the de-N-acetylated polysaccharide or de-N-acetylated oligosaccharide with an acryloylating reagent to form a  $\beta$ -propionated polysaccharide or a  $\beta$ -propionated oligosaccharide, and
- C) directly conjugating the  $\beta$ -propionated polysaccharide or the  $\beta$ -propionamido oligosaccharide to a protein to form the  $\beta$ -propionamido-linked polysaccharide-protein or  $\beta$ -propionamido-linked oligosaccharide-protein conjugate conjugate.
30. (withdrawn) The method of claim 29, wherein the de-N-acetylating reagent is a base or enzyme.
31. (withdrawn) The method of claim 29 wherein the de-N-acetylating reagent is selected from the group consisting of NaOH, KOH and LiOH.
32. (withdrawn) The method of claim 29, wherein the acryloylating reagent is selected from the group consisting of acryloyl chloride, acryloyl anhydride, acrylic acid and a dehydrating agent.
33. (withdrawn) The method of claim 29, wherein the polysaccharide or oligosaccharide is obtained from bacteria, yeast, cancer cells or is chemically synthesized.

34. (withdrawn) The method of claim 29 wherein the polysaccharide or oligosaccharide is obtained from *Escherichia coli*, Meningococcus, Pneumococcus, Streptococcus, Neisseria, Salmonella, Klebsiella, or Pseudomonas.
35. (withdrawn) The method of claim 29 wherein the protein is selected from the group consisting of tetanus toxoid, diphtheria toxoid, a neisserial outer membrane protein, pneumolysoid, C- $\beta$  protein from group B Streptococcus and non-IgA binding C- $\beta$  protein from group B *Streptococcus*.
36. (withdrawn) The method of claim 35, wherein the protein is recombinantly produced.
37. (currently amended) A vaccine comprising the conjugate according to any one of claim 1 and claim 16, wherein said vaccine generates antibodies that are reactive against the bacteria, yeast or cancer cell from which the polysaccharide or the oligosaccharide was isolated ~~derived~~ to confer immunity to said cell.
38. (cancelled)
39. (currently amended) The vaccine according to claim 37 wherein the polysaccharide or oligosaccharide is isolated ~~derived~~ from bacteria ~~are~~ selected from the group consisting of *Escherichia coli*, Meningococcus, Pneumococcus, ~~Streptococcus~~, Streptococcus, Neisseria, Neisseria, ~~Salmonella~~, Salmonella, ~~Klebsiella~~, Klebsiella, and *Pseudomonas* Pseudomonas.



40. (previously presented) The vaccine according to claim 37 further comprising a second immunogen in combination with the polysaccharide-protein conjugate or the oligosaccharide-protein conjugate, said second immunogen selected from the group consisting of diphtheria-tetanus-pertussis (DTP), diphtheria-tetanus-acellular pertussis (DTaP), tetanus-diphtheria (Td), diphtheria-tetanus-acellular pertussis-*Haemophilus influenzae type b* (DTaP-Hib), diphtheria-tetanus-acellular pertussis-inactivated poliovirus-*Haemophilus influenzae type b* (DTaP-IPV-Hib), and combinations thereof.
41. (withdrawn) A method of immunizing a mammal against a disease causing organism or disease causing cell comprising administering to the mammal an immunizing amount of the vaccine according to claim 37.
42. (withdrawn) A method of immunizing a mammal against *Streptococcus pneumoniae* comprising administering to the mammal an immunizing amount of the vaccine according to claim 37.
43. (withdrawn) A method of immunizing a mammal against Group B *Streptococcus* comprising administering to the mammal an immunizing amount of the vaccine according to claim 37.

44. (withdrawn) A method of immunizing a mammal against Group B *Neisseria meningitidis* comprising administering to the mammal an immunizing amount of the vaccine according to claim 37.
45. (withdrawn) A method of immunizing a mammal against Group C *Neisseria meningitidis* comprising administering to the mammal an immunizing amount of the vaccine according to claim 37.
46. (withdrawn) A method of immunizing a mammal against *Haemophilus influenzae* type B comprising administering to the mammal an immunizing amount of the vaccine according to claim 37.
47. (withdrawn) A method of eliciting an antibody response to a polysaccharide or an oligosaccharide in a mammal comprising administering an effective amount of the conjugate according to any one of claim 1 and 16.
48. (withdrawn) An immunoglobulin or antigen-binding fragment thereof produced according to the method of claim 47.
49. (withdrawn) The immunoglobulin according to claim 48, selected from the group consisting of IgG antibody, IgM antibody, IgA antibody and combinations thereof.

50. (withdrawn) The immunoglobulin according to claim 49, wherein the antibody is an isolated IgG.
51. (withdrawn) An isolated antibody or antigen binding fragment thereof elicited in response to the  $\beta$ -propionamido-linked polysaccharide-protein conjugate or  $\beta$ -propionamido-linked oligosaccharide-protein conjugate according to any one of claim 1 and 16, said antibody or antigen fragment thereof specifically immunoreactive with N-propionated polysaccharide or N-propionated oligosaccharide and immunoreactive with a native N-acetylated polysaccharide from which the  $\beta$ -propionated polysaccharide or  $\beta$ -propionated oligosaccharide was obtained.
52. (withdrawn) The antibody or antigen binding fragment thereof according to claim 51 wherein the native N-acetylated polysaccharide is obtained from bacteria, yeast, cancer cells, or is chemically synthesized.
53. (withdrawn) The antibody or antigen binding fragment thereof according a claim 52 wherein the polysaccharide is obtained from *Escherichia coli*, Meningococcus, Pneumococcus, Streptococcus, Neisseria, Salmonella, Klebsiella, or Pseudomonas.
54. (withdrawn) The antibody or antigen binding fragment thereof according to claim 51 wherein the antibody is recombinantly produced.

55. (withdrawn) A method of passive immunization against a disease causing organism or disease causing cells comprising administration of an effective amount of the immunoglobulin or antibody according to claim 48, said amount is sufficient to inhibit or kill the disease causing organism or disease causing cells.
56. (withdrawn) The method of passive immunization according to claim 55 wherein the immunoglobulin is an isolated IgG antibody or antigen binding fragment thereof.
57. (withdrawn) The method of passive immunization according to claim 55 wherein the immunoglobulin is an isolated IgM antibody or antigen binding fragment thereof.
58. (withdrawn) The method of passive immunization according to claim 55 wherein the immunoglobulin is an isolated IgA antibody or antigen binding fragment thereof.
59. (currently amended) The conjugate according to claim 1, wherein the polysaccharide or the oligosaccharide is at least ~~about~~ 95% N-acryloylated.
60. (previously presented) The conjugate according to claim 16, wherein the N-deacetylated polysaccharide or the N-deacetylated oligosaccharide is N-acryloylated by at least 95%.
61. (previously presented) The conjugate according to any one of claim 1 and claim 16, wherein the protein is a bacterial protein selected from the group consisting of tetanus toxoid, diphtheria toxoid, cholera toxin subunit B, *Neisseria meningitidis* outer

membrane proteins, pneumolysoid, C- $\beta$  protein from group B Streptococcus, *Pseudomonas aeruginosa* toxoid, and pertussis toxoid.

62. (withdrawn) A method of passive immunization against a disease causing organism or disease causing cells comprising administration of an effective amount of the immunoglobulin or antibody according to claim 51, said amount is sufficient to inhibit or kill the disease causing organism or disease causing cells.
63. (withdrawn) The method of passive immunization according to claim 62 wherein the immunoglobulin is an isolated IgG antibody or antigen binding fragment thereof.
64. (withdrawn) The method of passive immunization according to claim 62 wherein the immunoglobulin is an isolated IgM antibody or antigen binding fragment thereof.
65. (withdrawn) The method of passive immunization according to claim 62 wherein the immunoglobulin is an isolated IgA antibody or antigen binding fragment thereof.
66. (previously presented) The conjugate of any one of claim 1 and claim 6, wherein the polysaccharide or the oligosaccharide is a capsular or cell surface polysaccharide or oligosaccharide.